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16. ABSTRACT

Young soft shell clams, Mya arenaria, were exposed to subacute concentrations of No. 2 fuel oil-in-water emulsions under simulated winter (4°C) spill conditions. A pattern of accumulation and discharge of petroleum constituents, an experimental depuration time (biological half life, TB50), and a potential transport mechanism of aromatic compounds from the fuel oil to the clams were experimentally determined. The clams were exposed to single dose concentrations of 10, 50, and 100 ppm of No. 2 fuel oil-in-water emulsions for 28 days. Clams accumulated the greatest amount of hydrocarbons within one week after the initial exposure. The accumulated hydrocarbons decreased each week as the hydrocarbon content of the water decreased. Mass spectrometric analysis determined that the principle compounds accumulated and retained after 3 weeks of oil exposure were monomethyl, dimethyl and trimethylnaphthalene isomers. Depletion of oil from the water column and accumulation and discharge of fuel oil constituents appeared to involve a mucus-oil complex formation by the clam.

During the depuration period, accumulated hydrocarbons were rapidly, although incompletely discharged. At the end of the depuration period, many of the hydrocarbons present in the clam were dimethyl and trimethylnaphthalene isomers. The biological half lifes calculated were: 10 ppm (50 days); 50 ppm (11 days); 100 ppm (13.5 days).

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CHAPTER 32

THE ACCUMULATION AND DEPURATION OF NO. 2 FUEL OIL BY THE SOFT SHELL CLAM, *Mya arenaria* L.

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Abstract

Young soft shell clams, *Mya arenaria*, were exposed to subacute concentrations of No. 2 fuel oil-in-water emulsions under simulated winter (4°C) spill conditions. A pattern of accumulation and discharge of petroleum constituents, an experimental depuration time (biological half-life, TB_{50}), and a potential transport mechanism of aromatic compounds from the fuel oil to the clams were experimentally determined. The clams were exposed to single dose concentrations of 10, 50, and 100 ppm of No. 2 fuel oil-in-water emulsion for 28 days. Clams accumulated the greatest amount of hydrocarbons within one week after the initial exposure. The accumulated hydrocarbons decreased each week as the hydrocarbon content of the water decreased. Mass spectrometric analysis determined that the principle compounds accumulated and retained after three weeks of oil exposure were monomethyl, dimethyl, and trimethylnaphthalene isomers. Depletion of oil from the water column and accumulation and discharge of fuel oil constituents appeared to involve a mucus-oil complex formation by the clam.

The depuration period was determined when the clams were transferred to an uncontaminated system for 14 days subsequent to the 28 day oil exposure. Accumulated hydrocarbons were rapidly, although incompletely discharged. At the end of the depuration period, mass spectrometric analysis revealed that many of the hydrocarbons present in the clams were dimethyl and trimethylnaphthalene isomers. The biological half-lifes (TB_{50}) calculated were: 10 ppm (50 days); 50 ppm (11 days); 100 ppm (13.5 days).

Key words: *Mya arenaria*, oil accumulation, depuration, retention, No. 2 fuel oil, aromatic hydrocarbons, oil emulsion

Introduction

Chemical analyses have shown that various components of petroleum oils are accumulated within marine invertebrates. Blumer et al (1970), Ehrhardt (1972), Clark and Finley (1973) and Farrington and Quinn (1973) have reported the occurrence of petroleum oils in bivalve molluscs sampled from the environment. These molluscs were sampled from areas which had either been exposed to oil spills or from areas considered industrially contaminated. Laboratory studies of the accumulation of petroleum oils and oil fractions by mussels or oysters were investigated by Lee et al (1972), Blaylock et al (1973) and Stegeman and Teal (1973). Their work indicated that various fractions of petroleum oils can be accumulated and retained by mussels and oysters.

Oil spillage continues to be a serious problem in coastal and estuarine waters. Field studies of spills and their effects on *Mya arenaria* were documented by Thomas (1973) and Dow and Hurst (1975), and the occurrence of oil in *Mya arenaria* subsequent to environmental contamination was reported by Zitko (1971) and Scarratt and Zitko (1972).

Soft shell clams, *Mya arenaria*, occur frequently in areas receiving acute and chronic oil exposures and are often harvested for human consumption. This study was therefore performed to determine experimentally, a pattern of accumulation and discharge of petroleum constituents, a depuration time (biological half-life, TB_{50}), and a potential transport mechanism of aromatic constituents of the oil in soft shell clams exposed to No. 2 fuel oil-in-water emulsions. Few studies of oil accumulation and discharge have been performed at winter temperature conditions and experimentation was therefore performed at 4°C to simulate the winter state during which time spills and discharges were more likely to occur resulting from winter storms.

The digested material was transferred to a Teflon stoppered separatory funnel (1 liter) using distilled water (80 ml) and two portions (50 ml each) of hexane. The mixture was then shaken by hand for one minute. The two phases were then drained into separate flasks and the aqueous phase was returned to the separatory funnel using a hexane (50 ml) wash. The extraction and separation was repeated for a total of three times.

The combined extracts were washed (minimum 3 times) with aliquots (500 ml each) of distilled water and transferred to an Erlenmeyer flask (300 ml) using additional hexane. Water was removed from the extract by adding 2-3 gm of anhydrous Na_2SO_4. The extracts were then concentrated to approximately 40 ml under a stream of filtered nitrogen in a warm water bath (50°C) and transferred to a conical flask (50 ml) where concentration was continued to a final volume of 2 ml.

The concentrated extract was placed on a glass column (25 cm length x 1.0 cm I.D.). The column was packed with 6.5 cm of 5% water deactivated silica gel (100-200 mesh, Grade 923, Davison Chemical, Baltimore, Md.). Another 6.5 cm of 5% water deactivated alumina (Neutral, 90-20 mesh, Brockman Activity 1: Fisher Chemical Co.) was layered over the silica. Both the silica gel and alumina were activated at 110°C for 24 hours and then deactivited prior to column packing. Sand was layered over the alumina (0.5 cm). When packed, the column was rinsed with one column volume of hexane.

The residual concentrate was charged onto the column and then elutriated with 50 ml hexane. Subsequent analysis demonstrated that all petroleum hydrocarbons in the sample had passed through. The column was rinsed with 50 ml of benzene but few hydrocarbons were found in this fraction and it was later discarded. The eluate was concentrated for gas chromatographic analysis to a volume of 0.1 ml under nitrogen.

The identification of sample hydrocarbons was achieved by comparison with gas chromatograms of hydrocarbon standards and of No. 2 fuel oil in hexane. A Perkin-Elmer (Model 900) gas chromatograph was employed, equipped with a flame ionization detector and a 6 ft stainless steel column packed with 8% Dexsil on 80/100 mesh of Chromosorb W. Standard operating conditions were as follows: carrier gas: N_2, 2 cc/min; detector: H_2, 20 cc/min; air: 40 cc/min; injector temperature: 200°C; detector temperature: 300°C; manifold temperature: 300°C; column temperature: 70°C with a two minute hold, and programmed at an increase of 8°C per minute to 300°C; injection sample: 0.1 microliter. Further identifications of compounds were accomplished by mass spectrometric analysis provided by the U.S. EPA (IERL-Ci), Edison, N.J.

Hydrocarbons were quantified by cutting and weighing the chromatograms. The total areas under the peaks were obtained by subtraction of the baseline area of the first week control measurement. It was assumed that the area under the first week control measurement would be most representative of background levels and therefore any material above this baseline were considered accumulated petroleum hydrocarbons. The sample peak areas were compared to that of an internal standard (octacosane, C_{28}) having a known concentration (2 µg/gm clam tissue).

Hydrocarbon quantification results were analyzed for regression. The regression analysis used the No. 1987A/ST3 package from the 700 series Standard Statistical Program, Wang Laboratories, on a taped program. The significance of the regressions were further analyzed by a t-test (Zar, 1974).

Results

Water quality appeared excellent throughout the 28 day period. The dissolved oxygen and ph remained optimum in all tanks and bacterial contamination was not apparent. Control animals were reactive to tactile stimuli and appeared to be feeding. Very little mortality occurred at all oil concentrations. The actual mortalities were: Controls - 0.0%, 10 ppm - 0.5%, 50 ppm - 2.0%, 100 ppm - 3.0%.

During the 14 day depuration period, the 10 ppm clams seemed to recover their tactile irritability, in comparison to the controls. The 50 and 100 ppm clams were sluggish and after shucking many smelled foul. Their visceral mass was less firm than controls and their color had paled from the normal gold-brown. Mortalities occurred in the clams previously exposed to the concentration of 50 and 100 ppm. The actual mortality at the end of the depuration period was: Control 0.0%, 10 ppm - 0.0%, 50 ppm - 18.3% and 100 ppm - 13.6%.

The sea water before all tests and the controls during the tests did not have detectable hydrocarbon contents. Within the oiled tanks, some of the oil emulsion had broken within

two hours after addition. Much of the oil emulsion and water soluble fraction remained in the water column as indicated in Table 1. One week later, the detectable hydrocarbon concentrations in the water column of the oiled tanks were similar. Much of the oil was bound in mucus which adhered to the glass cooling coils and tank walls or formed floating organic flocculent conglomerates. The IR spectra also showed the emergence of an apparent aromatic band at 3030 cm^{-1}. The hydrocarbon peaks at 2930 cm^{-1} often masks the aromatics. Apparently, the water soluble aromatics of the fuel oil were becoming unmasked as more of the non-aromatic hydrocarbons became dissipated from the water column. The Week 2 data were similar to those of Week 1 and a peak emerged at ca. 3000 cm^{-1}.

TABLE 1. Hydrocarbon concentration (ppm) in the water column during the 28 day exposure period

Aquaria	Time 0*	Week 1	Week 2	Week 3	Week 4
Control	0	0	0	0	0
10 ppm	4.3	1.31	0.56	0.37	0
50 ppm	43.7	1.04	0.71	0.37	0.29
100 ppm	60.7	1.52	0.78	0.32	0.47

*The Time 0 sample measurement was made two hours after addition of the emulsified oil.

By Week 3, the measurable hydrocarbon content had dropped further and in the 10 and 50 ppm tank samples the possible aromatic peak became less apparent. The 100 ppm tank sample still had a strong peak at 3030 cm^{-1}. At the end of the fourth week, the hydrocarbon content in the tanks had fallen to almost trace concentrations and peaks were becoming indistinguishable.

The tanks used in the oil exposure experiments were drained after the fourth week. There was an extremely strong odor of No. 2 fuel oil, especially in the 100 ppm tank. As the mucus was wiped off the sides and cooling oils, the odor became readily apparent in all the oiled tanks. This indicates that much of the oil was bound by the mucus which had settled on the cooling coils and sides of the tanks. The mucus-oil complex formation may have aided in the dissipation of hydrocarbons from the water column. The mechanism of the binding of oil in mucus by the clams has been documented (Stainken, 1975). Mass spectrometric analysis demonstrated that the mucus contained predominantly dimethyl and trimethylnaphthalenes with paraffins, mainly in the C-14 and C-15 regions. Figure 1 illustrates the gas chromatograms of No. 2 fuel oil and an extract of hydrocarbons from a mucus sample. A close correlation of peak heights is readily noticeable. The chromatogram pattern of the mucus is similar to No. 2 fuel oil but some of the lower boiling compounds are missing.

During the depuration period, measurable amounts of hydrocarbons in the aquaria water were not detected. There was an emergence of a peak at 3000 cm^{-1} during the second week in the experimental tanks.

Gas chromatograms for the control clams are contained in Fig. 2. The basic pattern for the normal control clams remained unchanged during the experiment. The chromatogram for Week 1 was chosen as a baseline for the exposed animals to compare the amount of No. 2 fuel oil hydrocarbons accumulated. The chromatograms for the clams exposed to 10 ppm No. 2 fuel oil are illustrated in Fig. 3. The chromatograms A-C show a characteristic well defined pattern of peaks. Mass spectrometric analysis of the third weeks sample (chromatogram C) revealed that the bulk of the chromatogram consisted of monomethyl, dimethyl and trimethylnaphthalenes. By the fourth week, the resolution of the peaks had diminished and the chromatograms of the clams placed in fresh sea water would superficially indicate there were few hydrocarbons present. However, mass spectrometric analysis revealed that the sample (Chromatogram F, Fig. 3) contained dimethyl and trimethylnaphthalenes at the end of the depuration period.

Figure 4 depicts the chromatograms from the clams exposed to 50 ppm of No. 2 fuel oil emulsion. The same pattern of peaks as those of the 10 ppm is present. The peaks have much higher resolution and are still apparent even after the depuration period. A higher attenuation (10x64) had to be used because the samples contained a much higher hydrocarbon concentration. Mass spectrometric analysis of the Week 3 sample revealed that most of the sample was comprised of monomethyl, dimethyl and trimethylnaphthalenes. Mass spectrometric analysis of the clam sample at the end of depuration (Chromatogram F, Fig. 4) showed that the sample was composed of alkyl benzenes, monomethyl, dimethyl and trimethylnaphthalenes. The chromatogram of Week 1 control was drawn under chromatograms E and F, Fig. 4, to illustrate the quantity of petroleum hydrocarbons retained by the clams.

Chromatograms of clams exposed to a concentration of 100 ppm No. 2 fuel oil are illustrated in Fig. 5. The same recurrent pattern is readily visible and the peaks have sharper resolution. Mass spectrometric analysis of the week 3 sample showed it to consist of naphthalene, monomethylnaphthalene, a mixture of dimethyl and trimethylnaphthalene isomers, phenanthrene, and C_{12} and C_{14} paraffins. Mass spectrometric analysis of the clam sample after the two week depuration (Chromatogram F, Fig. 5) confirmed that the sample consisted of naphthalene, a mixture of dimethyl and trimethylnaphthalene isomers and no paraffins.

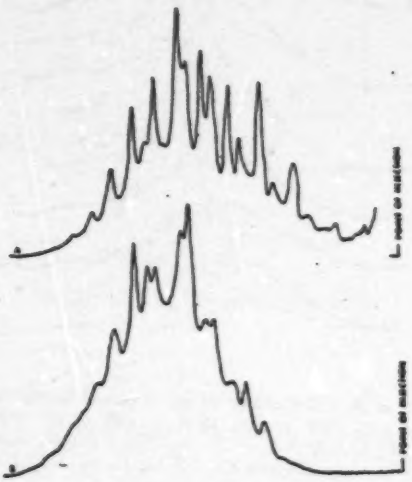

Fig. 32.1 Gas chromatograms
(A) 8 ppm #2 FUEL OIL IN HEXANE, ATTENUATION 1x128
(B) CARBON TETRACHLORIDE EXTRACT OF CLAM MUCUS, ATTENUATION 10x1024

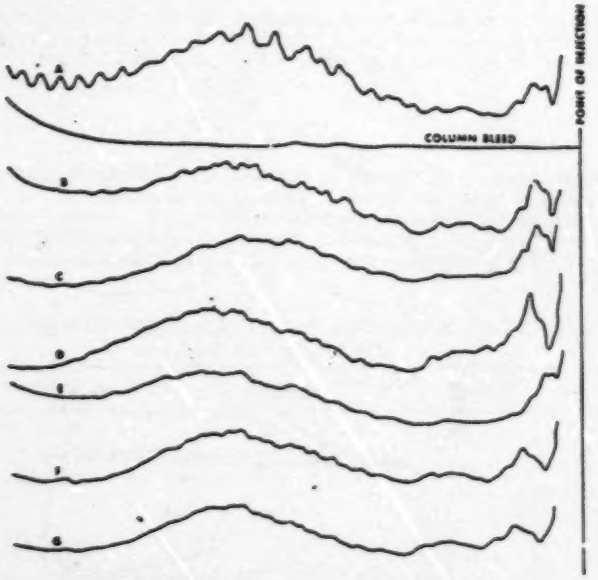

Fig. 32.2. Control animal gas chromatograms.
(A) TIME 0, WITH COLUMN BLEED DRAWN BELOW; (B) WEEK 1; (C) WEEK 2; (D) WEEK 3;
(E) WEEK 4; (F) CLAMS IN FRESH SEA WATER, WEEK 1; (G) CLAMS IN FRESH SEA WATER, WEEK 2
ALL CHROMATOGRAMS AT 1x128 ATTENUATION

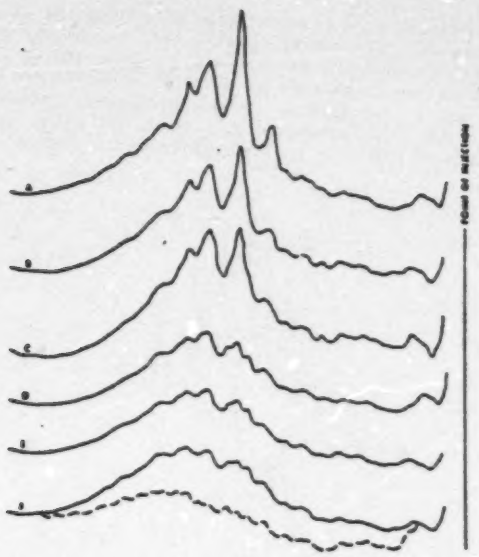

Fig. 32.3. Gas chromatograms clams exposed to 10 ppm #2 Fuel Oil.
(A) WEEK 1; (B) WEEK 2; (C) WEEK 3; (D) WEEK 4; (E) CLAMS PLACED IN FRESH SEA WATER, WEEK 1;
(F) CLAMS PLACED IN FRESH SEA WATER, WEEK 2, WITH CONTROL WEEK 1 DRAWN AS BASE LINE
ALL CHROMATOGRAMS AT 1x128 ATTENUATION

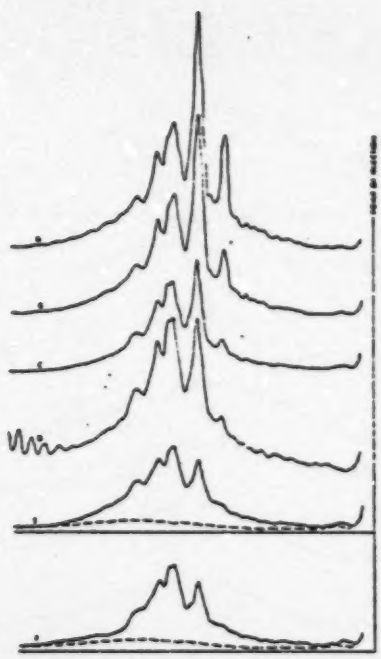

Fig. 32.4. Gas chromatograms clams exposed to 50 ppm #2 Fuel Oil.
(A) WEEK 1; (B) WEEK 2; (C) WEEK 3; (D) WEEK 4; (E) CLAMS PLACED IN FRESH SEA WATER.
WEEK 1, WITH CONTROL WEEK 1 AS BASELINE; (F) CLAMS IN FRESH SEA WATER, WEEK 2,
WITH CONTROL WEEK 1 AS BASELINE
ALL CHROMATOGRAMS AT 10x64 ATTENUATION

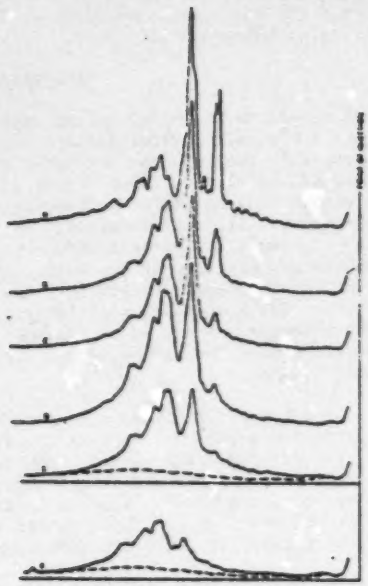

Fig. 32.5 . Gas chromatograms clams exposed to 100 ppm #2 Fuel Oil.
(A) WEEK 1; (B) WEEK 2; (C) WEEK 3; (D) WEEK 4; (E) CLAMS PLACED IN FRESH SEA WATER, WEEK 1, WITH CONTROL WEEK 1 AS BASELINE (F) CLAMS IN FRESH SEA WATER, WEEK 2, CONTROL WEEK 1 AS BASELINE
ALL CHROMATOGRAMS AT 10x64 ATTENUATION

The results of quantification of the gas chromatograms are listed in Table 2. Results are expressed as micrograms of petroleum hydrocarbons/gram clam sample. After one weeks exposure to oil, the values diminish with time. The sudden upsurges in Week 3 at 10 ppm and in Week 4, of the 50 and 100 ppm are unexplained.

Experimental concentration factors were calculated for the first week of exposure from the data in Table 1 and 2. The hydrocarbon concentration of the water column, Week 1, was subtracted from the Time 0 value. This net hydrocarbon concentration in the water column was then divided into the hydrocarbon value accumulated by the clam after the first week of oil exposure. The computed concentration factors were: 10 ppm - 8.5, 50 ppm - 3.3, 100 ppm - 1.8.

Table 2. Quantification of petroleum hydrocarbons (micrograms per gram of clam tissue).

	10 ppm	50 ppm	100 ppm
Week 1	27.3	143.1	109.3
Week 2	22.9	120.5	99.2
Week 3	31.6	97.0	90.7
Week 4	17.3	146.8	117.5
Week 5 (depuration)	12.9	66.8	83.6
Week 6 (depuration)	14.9	55.3	57.0

The biological half lifes (TB_{50}) or residence times were calculated using a regression analysis of the depuration period and plotting on semilog paper. The fourth weeks data were calculated as Time 0 for the depuration period. The calculated TB_{50} were: 10 ppm - 50 days; 50 ppm - 11 days; 100 ppm - 13.5 days.

Discussion

The concentrations of oil in the water column began to decrease several hours after the addition of the oil emulsion. Several factors were probably responsible for this. Much of the oil was apparently removed from the water column by the mucociliary feeding and ejection mechanisms of the clams. Large masses of mucus were ejected from the clams and were accumulated on the cooling coils. Subsequent chemical analysis revealed a large content of oil in the mucus. It is presumed that as the clams filtered water, they were exposed primarily to naphthalene derived compounds. Earlier investigators reported that fuel oil can form droplets and micelles in sea water, and that the major water soluble components were the aromatic compounds, particularly the naphthalenes (Boylan & Tripp, 1971; Boehm & Quinn, 1974). The accumulation of large amounts of oil and aromatic molecules in the mucus may be a potential mechanism of concentrating and disseminating petroleum hydrocarbons in the environment. The mechanism for the formation of an oil-mucus complex was discussed by Stainken (1975).

After Time zero, the oil rapidly disappeared from the water column. The amount of dissolved compounds attained a peak measured in the first week, and then decreased to an equilibrium value for several weeks ranging from 0.5-0.29 ppm. Analysis of mucus and clam tissue indicated that naphthalenes and methyl substituted naphthalenes were the apparent predominant components in the water. This is further substantiated by other reports (Frankenfeld, 1973; Anderson, et al., 1974) which indicate that the water soluble fractions of oils often contain aromatic compounds, particularly naphthalenes.

The peak accumulation of petroleum derived hydrocarbons by the clams was reached one week after exposure, followed by a gradual loss in accumulated hydrocarbons. The bulk of the material accumulated were naphthalenes and methyl substituted naphthalene isomers. Other aromatics were detected and accumulated but the naphthalenes were the most persistent. The results of this study are comparable to those of Vaughan (1973). He reported that oysters exposed to No. 2 fuel oil often accumulated methyl and dimethyl naphthalenes. Other studies also indicate that aromatic hydrocarbons may be accumulated by bivalves (Ehrhardt, 1972; DiSalvo, et al, 1973.

Paraffins or aliphatics were not detected in clam tissue in this study, except in the higher oil exposures. The aromatic compounds constituted a much greater percentage of the total oil extracted from the clams. The extracted concentrations of aromatics from the clams were also much greater than the original diluted concentrations when added at Time 0 as part of the fuel oil emulsion. Similar results of Stegeman and Teal (1973) demonstrate that a high aromatic content in the organism need not be the result of a high aromatic content in the contaminating oil. This phenomena is probably due to the greater solubility of the aromatic compounds (Anderson, et al., 1974; Boehm and Quinn, 1974).

The greater uptake of hydrocarbons by clams exposed to 50 ppm may be due to a dose dependent narcosis. Galtsoff, et al., (1935) reported that water soluble substances of crude oil could produce anaesthetic effects on the ciliated epithelium of the gills. The 100 ppm oil concentration may have reduced the filtration activity of the clams below that of the clams exposed to 50 ppm. The reduced filtration rate may account for the animals exposed to the higher concentration (100 ppm) accumulating less oil than the animals exposed to the lower concentration (50 ppm).

Depuration of accumulated petroleum derived hydrocarbons actually began as the hydrocarbons decreased in the water column. When the clams were removed to fresh sea water, depuration proceeded rapidly for the first week. The calculated half-time for depuration ranged from 11-30 days. The retention of hydrocarbons at the end of the depuration period indicated that after oil exposure, depuration will proceed rapidly to a low concentration after which depuration proceeds slowly. Other investigators have reported similar findings in other bivalve species (Lee, et al., 1972; Stegeman & Teal, 1973; Vaughan, 1973). However, the actual retention of oil by Mya arenaria in this study is further substantiated by the observations of Thomas (1973) in which clams were observed to eject oil for at least one month after an oil spill.

Oil retention can have several deleterious effects. Long term low level petroleum hydrocarbon contamination may interfere or weaken the ability of the clam to withstand further environmental stresses such as those of temperature, salinity, spawning, disease and insult from other contaminants. Furthermore, oil retained in the sediment and clams may also be passed on to predators, including man.

Summary

1. Clams accumulate large amounts of petroleum derived hydrocarbons after initial exposure to petroleum (No. 2 fuel oil). The amount and the type of petroleum hydrocarbon accumulated are related to the initial dose, time following initial exposure and the relative "solubilities" of the various hydrocarbon components. Naphthalene and methyl substituted naphthalene isomers (mono, di, tri) are the most common compounds concentrated by the clams. Occasionally, some alkyl benzenes, phenanthrenes and paraffins in the C-12 and C-14 region are accumulated.

Depuration by soft-shell clams of accumulated petroleum hydrocarbons may actually begin as soon as hydrocarbon levels drop in the water column. When clams are placed in fresh hydrocarbon free sea water, depuration proceeds rapidly within the first week after which the rate slows. Dimethyl and trimethylnapthalenes were still present in the clams after two weeks in fresh sea water.

2. Chemical analysis of clam mucus extract demonstrated that clams can concentrate and ingest or release to the environment extremely large amounts of petroleum hydrocarbons by means of their mucus - oil binding mechanism. Analysis of petroleum compounds present in the mucus revealed them to be methyl substituted naphthalenes and paraffins, all compounds similar to those which the clams accumulate. Types of chemical compounds accumulated or bound in the mucus are probably related to differential solubilities of petroleum constituents.

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